REMARKS

Applicants appreciated Examiner's withdrawal of certain objections and rejections from the previous office action.

Specification objections

Applicants hereby provide substitute page 23 to remove the large blank space on page 23.

Applicants will file an information disclosure statement, pursuant to 37 CFR 1.98(b) and on a proper from PTO-892, listing all relevant patents and publications listed in the specification.

Claim Objections

The claims have been amended to remove superfluous wording.

New-Claim Rejections under 35 USC § 112, Second Paragraph: Indefiniteness

Claims 5 and 39 (and 40-49 depending therefrom), were rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner alleges that claims 5 and 39 are not clear and are confusing because it is not clear how increasing the amount of a gene product produces a xanthan composition.

Applicants respectfully direct the Examiner to at least pages 3, 4, and 8, and Tables 1, 3, and 4, of the specification. One skilled in the art, upon reading the claims and at

least these pages of the specification, would understand that over-expressing the genes described would result in a xanthan composition having the characteristics described in the claims.

The Examiner next rejects claims 5, 39 (40-49 depending therefrom) and 50 (51 and 53-58 depending therefrom) as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as their invention. Specifically, the Examiner states that it is not clear whether the phrase ". . . *Xanthomonas campestris* (XWCM1/pBBR5BC) . . . is synonymous with a 'specific strain of *Xanthonmonas campestris'* or does it include any *Xanthomonas campetris* including mutants thereof.

Applicants believe that the current amendments to the claims address this issue and clarify that the claims are intended to cover any *Xanthomonas campestris* strain, and mutant strains thereof, provided the strains have multiple copies of wild type *gumB* and wild type *gumC*.

Claims 5, 39 (40-49 depending therefrom) and 50 (51 and 53-58 depending therefrom) were rejected as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as their invention. These claims recite the phrase " . . . *Xanthonmonas campestris* (XWCM1/pBBR5BC) . . . is [this] a limitation of the claim or merely exemplary.

Applicants believe that the current amendments to the claims address this issue and clarify that this was meant to be merely exemplary.

New-Claim Rejections under 35 USC § 112, Second Paragraph: Enablement

Claims 5 and 39-51 and 53-58 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The examiner recites that the claims contain

"subject matter which was not described in the specification in such a way as to enable one skilled it the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Claims 5, 39-42, 46, 47 and 50-56 recite plasmid pBBR5BC comprised in specific strain of *Xanthomonas comapestis* XWCM1." Office action, page 4.

The Examiner asserts further:

> It is apparent that plasmid pBBR5BC comprised in specific strain of *Xanthomonas campestris* is required to practice the claimed invention. As such the biological material must be readily available or obtainable by a repeatable method set forth in the specification, or otherwise readily available to the public. If it is not so obtainable or available, the requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the plasmid pBBR5BC comprised in specific strain of *Xanthomonas campestris*. The claimed plasmid sequences are not fully disclosed, nor have all sequences derived for their construction and the specific strain been shown publicly available.

The sequences of *Xanthomonas campestris* genes *gumB, gumC, gumD, gumE, gumF, gumG, gumH, gumI, gumJ, gumK, gumL and gumM* were deposited in GenBank, Accession number U22511. The sequence of "gumA" was also deposited in U22511, but is now considered two genes, *ihfA* and *orfX*.

A correction to the sequence of *gumB* was published by M. V. Ielmini, F. Katzen and L. Lelpi in 2001, Accession number AF427012. In addition, genomic sequences have been determined for two other closely related strains. *Xanthomonas campestris* pathovar *campestris* strain, ATCC 33913 genome sequence has been published, included the *gum* genes, Accession numbers AE012357, AE012356, AE012355. The genome sequence of *Xanthomonas campestris* pv. *campestris* strain 8004 has been deposited as CP000050.1 The vector used for construction of pBBR5-BC is pBBR1MCS-5, GenBank accession No. U25061.

Applicants believe that the current amendments to the claims and the information regarding availability of sequences address this rejection and render it moot.

Maintained claim Rejections 35 USC 103

Claims 5 and 39-51 and 53-58 were again rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Hassler et al., (1990), Becker et al., (1998), Katzen et al., (1998) and further in view of Feinbaum R (1998).

The Examiner specifically asserts:

> 1) at the outset examiner would like to point out that the amended claims are directed to over-expression of *gumB* and *gumC* in a mutant (undefined genetic lesion) of *Xanthomonas campestris* XWCM1 and not the wild type strain.
> 2) The cited reference indeed provide the teaching, suggestion and motivation for the instant invention i.e., method for the production of xanthans with increased viscosity by selectively increasing the gene product of *gumB* and *gumC*.
> (i) Becker et al, as previously cited (pages 10-11 of FOAM dated 2/21/2007) with respect to the biochemical assignments for all the genes involved in the xanthan biosynthetic pathway in *Xanthomonas campestris*; specifically the reference teaches a) that the gene products of *gumB* and *gumC* are involved in the terminal states of xanthan biosynthesis and regulate the xanthan export and polymerization of the molecule and b) Becker et al., had envisioned that mutants (not directly involved in the biosythesis of xanthans) that produced xanthans with increased viscosity as a result of increased pyruvate content can be further improved by overexpressing genes of xanthan biosynthetic pathway (page 149, second paragraph).
> (ii) Examiner would like to point out that Hassler's reference also provides knowledge regarding mutants not directly involved in the biosynthetic pathway, but enzymes that are involved in adding either acetyl or pyruvate moieties to the mannose residues in the repeating structural units of xanthan polymer, this "decoration" of mannose moieties either with acetyl or pyruvate moieties determines the viscosity of xanthan. Examiner would like to draw

the attention of the applicants' to the following mutants described by Hassler et al., in the Introduction section (pages 182-183, Table: 1 and Figure 2), specifically the following mutants: "Mutants were constructed that comprises all possible combinations of mutations in genes F, G and L . . . Analysis of the properties of this family of variant xanthans allows investigation of the effect of the individual modifications on polymer properties."
(iii) Katzen et al. also provide evidence that inactivation of *gumB* or *gumC* or *gumE* in the wild-type strain was lethal (as these genes are involved in polymerization and export of xanthan). Absence or deficiency of one of the *gumB* or *gumC* or *gumE* would disrupt the polymerization process impairing accumulation of lipid-linked intermediates and elevated levels of these compounds might prove toxic to the cells (page 1615, columns 1-2).

. . .

Given the knowledge of biosynthetic pathway for xanthan synthesis, especially involvement of *gumB* (polysaccharide translocation) and *gumC* (degree of polymerization), a skilled artisan would be motivated to increase specifically the *gumB* and *gumC* gene products involved in the terminal states of xanthan biosythesis that regulate the xanthan polymerization and export in a strain tailored to produce xanthan with increased viscosity (examiner would also like to emphasize that the *gumB* and *gumC* genes are overexpressed in a mutant of undefined genetic lesion in the instant invention). Said method of producing xanthan polymer preparation having increased viscosity will entail/encompass; increasing the activity of genes involved in the polymerization i.e., *gumB*, as *gumB* is clearly involved in the polymerization of xanthan determining the size and length of the xanthans (as taught be Becker et al.) and also genes involved in the transport of xanthan i.e., *gumC*, as one would like to efficiently transport the xanthan with increased viscosity that accumulates intracellularly and potentially detrimental to the engineered microorganism (as taught by Hassler et al. and Katzen et al). Therefore, in engineered cells that produce xanthans with increased viscosity, to achieve efficient polymerization, secretion and at the same time overcome the intracellular accumulation of xanthans with increased viscosity that could potentially result in toxic effects on the engineered cells, a skilled artisan would be motivated to increase the amount of *gumB* and *gumC* gene product. It is *prima facie* obvious to a skilled artisan to realize the strong nexus provided by the teaching

of the prior art and would be motivated to combine the teaching of cited references at the time of the instant invention with high likelihood of success for a method of producing xanthan polymers with high viscosity that involves increasing the amount of *gumB* and *gumC* gene products in engineered cells.

Applicants respectfully disagree with the Examiners arguments for the following reasons.

The works of Katzen *et al.* and of Becker *et al.* show that the products of four genes (*gumB, gumC, gumE* and *gumJ*) are involved in steps occurring after the assembly of the lipid-linked pentasaccharide intermediates of xanthan. The exact biochemical steps catalyzed by these gene products, however, are not known and therefore were not known at the time of the invention. "The export mechanism of the polysaccharide remains to be determined but it might be associated to the polymerization event." Becker *et al*, page 146. The terminal stages of xanthan biosynthesis may include "flippase" activity (transfer of the lipid-linked pentasaccharide intermediate from the inner membrane facing the cytoplasm to the other side of the inner membrane facing the periplasm), polymerization of the pentasaccharide subunits, pore formation, transport through the outer membrane, and release from the cell, as is well known in the art. It is not known exactly where these four proteins function or which proteins determine the length of the xanthan polysaccharide chain before it is released from the cell. These four proteins and other proteins for xanthan biosynthesis (in particular, glucosyl-1-phosphate transferase or glycosyl transferase I, encoded by *gumD*) are likely to form a membrane-bound complex. Interactions between the proteins in this complex are likely to be important for functionality, as is well known in the art and discussed by Katzen et al., page 1615, with respect to GumB, GumC, and GumE.

Based on the predicted amino acid sequences, both GumB and GumC are membrane proteins; GumB is postulated to be an outer membrane protein and GumC is proposed to be in the cytoplasmic membrane. The report by Katzen *et al.,* on page 1615, speculates that "GumB might be involved in polysaccharide translocation, whereas GumC might be involved in determination of the degree of polymerization", and that "*gumE* product might be directly related to the polymerization of xanthan" (emphasis added). It should be noted that although *gumC* is homologous to the *exoP* gene of *Rhizobium meliloti* that affects the ratio of high molecular weight to low molecular weight polysaccharide (Katzen et al. page 1614), in *X. campestris*, low molecular weight xanthan has not been detected. Id., at 1615. No obvious function for GumJ has been determined, however, this protein is homologous to proteins involved in polysaccharide biosynthesis and export, and inactivation of this gene is lethal in a strain that is wild-type for xanthan synthesis. Id.

From the previous published works, it was not known and one in the art would not be motivated to specifically amplify *gumB* and *gumC* together (but not *gumB* or *gumC* individually or *gumB, gumC*, *gumD* and *gumE* together) to achieve increased length of the xanthan chains, resulting in higher low shear rate viscosity (LSRV). The present invention shows an increase in chain length with *gumB* and *gumC* amplified on a multicopy plasmid, whereas amplification of alternatively *gumB* or *gumC* on the same plasmid did not result in higher LSRV. Also, amplification of *gumB* and *gumC* together with *gumD* and *gumE* or amplification of all genes in the operon (*gumB* through *gumM*), resulted in lower LSRV. Thus, extra copies of both the *gumB* and *gumC* genes, relative to the other genes in the *gum* operon, are critical to achieve the observed increase in molecular chain length of xanthan, and this has not been disclosed in the prior art sufficiently to motivate one skilled in the art to make the claimed invention.

For all of the foregoing reasons, applicants assert that one skilled in the art would not be motivated to combine the cited references to selectively amplify only *gumB* and *gumC* together in a *Xanthomonas campestris* strain, as set forth in the newly amended claims.

Conclusion

Applicants believe that the above-mentioned amendments and arguments place the claims in condition for allowance or in better from for appeal. No new matter has been introduced.

Respectfully submitted,

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